4. ASSOCIATION BETWEEN NON-MEDICAL PRESCRIPTION OPIOID USE AND PHYSIOLOGICAL STRESS RESPONSES TO SOCIAL REJECTION[4]

4.1 Abstract

Non-medical prescription opioid use (NMPOU) recently increased dramatically especially in the U.S. Although chronic opioid use is commonly accompanied with deficits in social functioning and dysregulation of the hypothalamic-pituitary adrenergic (HPA) stress axis, little is known about the impact of NMPOU on responses to psychosocial stress. Therefore, we measured physiological responses of the autonomic nervous system and the HPA axis to social rejection using the Cyberball paradigm. We compared 23 individuals with NMPOU, objectively confirmed by hair and urine analyses, with 29 matched, opioid-naïve, healthy controls. As expected, heart rate variability (HRV) increased significantly during exclusion within controls, while in the NMPOU group only a trend in the same direction was found. However, HRV increase was robustly modulated by opioid craving indicating worse emotion regulation to social exclusion specifically in NMPOU individuals with high craving symptoms. In contrast, skin conductance levels (SCL) upon social exclusion revealed no group differences. Increased plasma levels of the adrenocorticotropic hormone (ACTH) and cortisol were found in the NMPOU group indicating a hyperreactivity of the HPA axis to social rejection. Behavioural data suggest that opioid users were aware of being excluded but less emotionally affected by the rejection. Moreover, NMPOU individuals reported a smaller social network size compared to controls. Present findings suggest that chronic NMPOU is associated with dysfunctional physiological responses to psychosocial stressors such as social rejection. Additionally, NMPOU goes along with an abnormal regulation of the parasympathetic nervous system specifically under opioid craving highlighting its potential importance in relapse prevention.

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4.2 Introduction

In the last decades, the misuse of prescription opioids (e.g., morphine, fentanyl, oxycodone, or codeine) increased dramatically. Especially in the U.S., non-medical prescription opioid use (NMPOU) has reached epidemic dimension with a past-year prevalence of 37.8% of which 12.5% fulfilled opioid misuse criteria. Accordingly, NMPOU-related deaths dramatically increased by 265% from 2012 to 2015 (Han et al., 2017; UNODC, 2017). Importantly, relapse after detoxification is higher for opioids than for any other drug with a relapse rate up to 91% (Smyth, Barry, Keenan, & Ducray, 2010) signifying a lack of effective long-term treatments for opioid abstinence. A crucial key factor to prevent relapse of drug addiction is functional social support (Ellis et al., 2004; Havassy et al., 1991), while opioid use is commonly accompanied with deficits of social functioning. Recently, we found inferior performances in understanding and recognising others' feelings and emotions of others in individuals with NMPOU compared to healthy controls, which is crucial for prosocial behaviour and interpersonal relationships (Kroll, Nikolic, Bieri, Baumgartner, Soyka, & Quednow, 2018a). Furthermore, the Brain Opioid Theory of Social Attachment (BOTSA), initially formulated by Panksepp et al. (1978a), proposed an association between the opioid system and social bonding including social rejection. In line with the BOTSA, μ-opioid receptor (MOR) agonists might induce feelings of social comfort and subsequently reduce affiliative behaviour. Findings in animal studies supported this assumption reporting relief from separation distress measured by reduced isolation calls and decreased affiliative behaviour such as social grooming after administration of MOR agonists. Accordingly, MOR antagonist increased separation distress and subsequently motivation to seek social contact in animals to counteract this negative effects (Machin & Dunbar, 2011). In line with these findings, a recent study in humans reported reduced feelings of connection with close others in the laboratory and also in day-to-day reports after administration of the MOR antagonist naltrexone (Inagaki et al., 2016). Furthermore, decreased activation of the endogenous μ-opioid system measured by pain tolerance was linked to smaller social network size (Johnson & Dunbar, 2016) and low baseline MOR availability was associated with avoidant attachment style using positron emission tomography (PET) supporting the BOTSA in humans (Nummenmaa et al., 2015). Further neuroimaging studies using PET and functional magnetic resonance imaging (fMRI) reported increased neuronal and MOR activity during social rejection specifically in the dorsal anterior cingulate cortex (dACC), amygdala, anterior insula, and prefrontal cortex (PFC). These brain areas are also associated with high MOR density and the affective pain system indicating a neuronal overlap of physical and social pain, i.e., social rejection, exclusion, or ostracism (Cacioppo, Frum, Asp, Weiss, Lewis, & Cacioppo, 2013; Eisenberger, 2015; Hsu et al., 2013; Woo, Koban, Kross, Lindquist, Banich, Ruzic et al., 2014). Social pain is commonly measured by the Cyberball paradigm in humans, a virtual ball-tossing game, where participants were told to play a computer game with two other players,

who are in reality controlled by the experimenter to ostensibly exclude or include the participant. (Williams & Jarvis, 2006). Apart from neurocortical activation during social rejection, social pain is also linked to social stress response of the autonomic nervous system (ANS) and the hypothalamic-pituitary adrenergic (HPA) axis. ANS response to social rejection was reported in healthy subjects indexed by heart rate deceleration and altered skin conductance level (SCL) (Kelly, McDonald, & Rushby, 2012; Moor, Crone, & van der Molen, 2010; van der Veen, van der Molen, Sahibdin, & Franken, 2014). Furthermore, increased cortisol levels after social exclusion were found indicating activation of the HPA axis (Blackhart, Eckel, & Tice, 2007; Zwolinski, 2008). However, stress response of the ANS and HPA axis to social pain is inconsistent and other studies were not able to replicate the reported findings or rather revealed contrary results (Bass, Stednitz, Simonson, Shen, & Gahtan, 2014; Jobst, Sabass, Palagyi, Bauriedl-Schmidt, Mauer, Sarubin et al., 2015; Zwolinski, 2012). Two recent studies from the same group investigated acute effects of the partial MOR agonist buprenorphine on psychosocial stress and reported decreased feelings of social rejection using the Cyberball task and attenuated cortisol levels after psychosocial stress induced by the Trier Social Stress Test (TSST) in healthy buprenorphine-administered participants (Bershad, Jaffe, Childs, & de Wit, 2015; Bershad et al., 2016). So far only one study investigated the impact of NMPOU on psychosocial stress processing: Back et al. (2015) reported no differences in heart rate (HR) and saliva cortisol levels between healthy controls and individuals with prescription opioid dependence after the TSST. However, in this study cortisol levels were measured in the morning, which is an unfavourable daytime to assess stress-related cortisol response due to its diurnal rhythm and ceiling effects (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999).

Although reported findings in animals and humans indicated that the μ-opioid system might play a crucial role in interpersonal behaviour and processing of social rejection, studies investigating social pain in individuals with chronic NMPOU are still lacking. Therefore, the aim of our study was to assess stress response to social rejection on a behavioural and physiological level in individuals with NMPOU compared to healthy matched controls. We collected behavioural, neuroendocrine, and electrophysiological data in order to measure the activity of the ANS and the HPA axis during and after the Cyberball paradigm. Furthermore, participants' social network size was assessed by a questionnaire. Based on reported dampening effects of acute opioid administration on social stress (Bershad et al., 2015; Bershad et al., 2016), we hypothesised that individuals with NMPOU are less affected by social rejection compared to controls reflected in reduced behavioural and physiological stress responses. In accordance with the BOTSA, we further assumed that individuals with NMPOU reveal a smaller social network size than controls due to decreased affiliative behaviour.

4.3 Methods

4.3.1 Participants

The sample consisted of 23 individuals with NMPOU and 29 opioid-naïve healthy controls matched for sex, age, years of education, and smoking status. General exclusion criteria were neurological disorders or head injuries, severe physical diseases (e.g., HIV, HCV, or diabetes), axis-I DSM-IV and DSM 5 psychiatric disorders (except for alcohol and nicotine use disorders), chronic pain disorder, recent emotional painful events (e.g., breakup of a relationship or death of a close friend/relative), and insufficient proficiency in German language. Substance use disorder and history of depression were no exclusion criteria for the NMPOU group, whereas participants showing any intravenous drug use or a history of street heroin dependence were excluded. The inclusion criterion for opioid users was NMPOU at least over the last six months. Participants were instructed to abstain from psychotropic substances for 72h and for 24h from alcohol. Furthermore, opioid users were asked to abstain from opioids on the testing day, or to take an adequate and minimised dose of opioids, if necessary, which solely removed withdrawal symptoms, to avoid measuring acute or withdrawal effects. Opioid use was objectively determined by urine and hair toxicology analyses using a semi-quantitative enzyme multiplied immunoassay method and liquid chromatography-tandem mass spectrometry, respectively (for technical details see Kroll et al, 2018). The study was approved by the Cantonal Ethics Committee of Zurich. All participants provided written informed consent and received compensation for their participation.

4.3.2 Procedure

The Structured Clinical Interview for axis-I DSM-IV Disorders (SCID-I), adapted for DSM-5 regarding substance use disorders, was carried out by trained psychologists at the beginning of the testing day (Wittchen et al., 1997). Substance use was assessed by means of a standardised and structured Interview for Psychotropic Drug Consumption (Quednow et al., 2004). Current opioid craving was assessed by a Numeric Rating Scale (NRS) from one (no craving) to ten (highest craving) and by the Objective Opioid Withdrawal Scale (OOWS) (Handelsman et al., 1987). Additionally, self-report questionnaires were applied to determine severity of nicotine dependence (Fagerström Test of Nicotine Dependence, FTND) (Heatherton et al., 1991) and depressive symptoms (Beck Depression Inventory, BDI) (Beck et al., 1961). Premorbid verbal IQ was assessed with a German vocabulary test– the Mehrfachwahl-Wortschatz-Intelligenztest (MWT-B) (Lehrl, 1999). After clinical assessments, an intravenous (i.v.) catheter was placed at the forearm vein of the non-dominant hand by a trained and certified psychologist approximately one hour prior to the first blood sample. Following the

Cyberball, a neuropsychological test battery was applied, whose results have been published elsewhere (Kroll et al., 2018a).

4.3.3 Behavioural assessments

Cyberball paradigm

All participants were seated in a soundproof experimental room with a distance of 60 cm to the screen, where the Cyberball game was played (Williams & Jarvis, 2006). Participants were told that the task assesses performances of mental imagery and therefore they should try to imagine the ball-tossing situation as realistic as possible. To increase the credibility of the game, we implemented a photo of each participant and of the two teammates, who were in reality members of our group, in the game and introduced them personally prior to the Cyberball. After the task started, participants were included in the game for about one minute and received the ball six times (10%). Subsequently, participants were excluded and did not receive the ball anymore for the next two minutes controlled by the computer. The total duration of the game was about three minutes (60 throws). To assess behavioural responses to social rejection, the Positive and Negative Affect Schedule (PANAS) (Watson, Clark, & Tellegen, 1988) was used before and after the Cyberball. Differences in positive (PA) and negative affect (NA) were measured on a five-point Likert scale. Furthermore, participants completed a questionnaire after the Cyberball where they were asked how excluded and included they felt on a nine-point Likert scale and how often they received the ball in percentage.

Social Network Questionnaire (SNQ)

Based on the social contact circle interview (Linden, Lischka, Popien, & Golombek, 2007), the SNQ evaluates the amount of social contacts in specific areas of life such as household, family, work or apprenticeship, friends, neighbours, (sport) clubs or unions, and others. Only direct contact over the last four weeks such as personal encounters, via telephone, email, or letter were asked. For calculating the total social network size, participants were told to name contacts only once within the SNQ to avoid double entries of contacts in different areas.

4.3.4 Physiological stress responses to social rejection

Electrophysiological responses of the ANS

Spontaneous fluctuation (SF) in skin conductance during the Cyberball was assessed as an index for sympathetic activity (Bach, Daunizeau, Kuelzow, Friston, & Dolan, 2011). Skin conductance was recorded on the thenar/hypothenar of the non-dominant hand using two 8mm disc Ag/AgCl cup electrodes (EL258, Biopac Systems Inc., Goleta, CA) and 0.5% NaCl gel (GEL101, Biopac Systems Inc.) (Hygge & Hugdahl, 1985). Skin conductance signal was amplified with an SCR coupler/amplifier (V71-

23, Coulbourn Instruments), digitised at 1000 Hz using Dataq card (DI-149, Dataq Inc., Acron, OH), and recorded with Windaq (Dataq Inc.) software. SF over the total Cyberball game and during exclusion (60 sec after Cyberball onset) was analysed by the Matlab toolbox for psychophysiological modelling, PsPM 4.0 (pspm.sourceforge.net) using two models: 1. classical skin conductance level (SCL) representing the mean signal over all epochs in μS and 2. dynamic causal modelling (DCM), which is a non-linear estimation of the number of SF with a detection threshold of 0.1 μS and provides the most sensitive indicator for SF (Bach et al., 2011).

During the Cyberball game, electrocardiogram (ECG) was applied to assess the beat-to-beat variability in heart rate mediated by the vagus nerve, better known as the vagally mediated heart rate variability (vmHRV) (Laborde, Mosley, & Thayer, 2017). The vmHRV provides an index of the parasympathetic nervous system (PNS) (Chapleau & Sabharwal, 2011; Malik, 1996). Cardiac activity was recorded via a three-lead ECG at 1000 Hz sampling rate with 45-mm, pregelled Ag/AgCl adhesive electrodes attached below the right clavicle, on the left side of the abdomen below the heart, and on the right side of the abdomen. Data were preamplified and 50 Hz notch-filtered with a Coulbourn isolated five-lead amplifier (LabLinc V75-11, Coulbourn Instruments) and recoded with Windaq (Dataq Inc.) software. ECG data were analysed using Kubios HRV Premium 3.0.2 (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014) providing the calculation of time- and frequency-domain indices of vmHRV, i.e., the root mean square of successive differences (RMSSD) and the high-frequency (HF) band (HF-HRV, 0.15-0.4 Hz) (Laborde et al., 2017). Artefact correction was conducted manually in Kubios, if necessary. Because parasympathetic effects are usually faster than sympathetic effects (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012) and vmHRV is associated with emotion regulation and adaptation to stressful events, we decided to split the ECG data into inclusion (50 sec) and exclusion condition (110 sec) and analysed them separately. Therefore, we were able to compare changes in vmHRV from inclusion to exclusion.

Neuroendocrine response of the HPA axis

Neuroendocrine assessments were conducted after midday as the afternoon was suggested as favourable for studying provoked stress response of neuroendocrine hormones (King & Liberzon, 2009) (for more details see Methods S1). Blood samples were taken from an i.v. catheter with exception of two participants in the NMPOU group because of needle phobia and problems with placing the i.v. catheter. In total, five blood samples per participant were collected about 20 minutes before (baseline, T1) and +10 (T2), +20 (T3), +30 (T4), and +60 (T5) minutes after psychosocial stress induced by the Cyberball task (Kirschbaum et al., 1999) using BD Vacutainer® containing Lithium Heparin. Plasma samples were stored at -80°C until analysis. After completion of the study, all

samples were analysed via immunoassays in a specialised laboratory at the Technical University of Dresden (Dresden LabService) to assess neuroendocrine stress parameters of the HPA axis i.e. adrenocorticotropic hormone (ACTH) and cortisol. ACTH data of one participant in the control group were not detectable due to haemolytic plasma samples. Single missing data in plasma cortisol and ACTH were estimated by calculating the mean of the flanked plasma values, if missing occurred between two values. If missing values occurred for the first or last sample, the average difference of the respective group between the first and the second or the fourth and fifth value was subtracted from the second or forth value of the individual, respectively.

4.3.5 Statistical Analyses

Statistical analyses were conducted using SPSS 23.0 software. Frequency data were analysed by means of Pearson's χ^2. Quantitative data were either analysed by independent t-tests or Mann-Whitney-U-tests, if normal distribution was not given. In order to analyse differences within groups, paired samples t-tests or Wilcoxon signed rank tests were applied in case of non-normal distribution. Additional analyses of covariance (ANCOVA) for all behavioural responses to social rejection and the social network size were conducted to control for age and sex distribution because of reported associations with prosocial behaviour (Beadle et al., 2015; Kret & De Gelder, 2012; Miller et al., 1991; Smith, Marcum, Boessen, Almquist, Hipp, Nagle et al., 2015).

Changes in the distribution of cortisol and ACTH over time were analysed using repeated measures ANOVA with *time* (5 time points) as within-subjects factor and *group* as between-subjects factor. Greenhouse-Geisser correction was used, if the assumption of sphericity was violated. Furthermore, area under the curve with respect to increase (AUC_i) was calculated for the hormone profiles based on Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). Furthermore, to control for baseline (T1) hormonal levels and body mass index (BMI) ANCOVAs with the respective covariates were conducted with the neuroendocrine data and HRV, respectively (Koenig, Jarczok, Warth, Ellis, Bach, Hillecke et al., 2014). Log-transformation was applied when data showed right-skewed distribution (log_{10}). Pearson's product-moment correlations within the NMPOU group were used to investigate associations between drug use severity and physiological stress responses. The confirmatory statistical comparisons were carried out on a significance level of $p<0.05$ (two-tailed) with exception of the correlation analyses, where $p<0.01$ (one-tailed) was applied in order to avoid alpha-error accumulation. Cohen's *d* effect size (Cohen, 1988) was calculated by the means and pooled standard deviations of both group.

Based on the recent study by Garland, Bryan, Nakamura, Froeliger, and Howard (2017) investigating changes in HRV between opioid-misusers and non-misusers in chronic pain patients, we calculated an a priori power analysis using G*power 3.1 (Faul, Erdfelder, Buchner, & Lang, 2009) assuming an effect size of $\eta^2_{partial}$=.07, α-error probability of 5%, power of 90%, and a correlation of 0.5 among repeated measures for the 2 x 2 mixed ANOVA within-between interaction suggesting a total minimum sample size of N=38.

Considering the differences of opioids' analgesic potentials, morphine equivalents (ME) for each opioid (self-report and hair concentration) was calculated based on ME conversion factors per mg of opioid previously reported (Kroll et al., 2018).

4.4 Results

4.4.1 Demographic characteristics and drug use

As previously reported (Kroll et al., 2018a), controls and individuals with NMPOU did not significantly differ in demographic variables (Table 1). However, individuals with NMPOU showed higher BDI scores, as it was shown for opioid users before (Ersche et al., 2006). Individuals with NMPOU mostly reported only mild opioid craving at a median of 3.00 (range 1-8). Severe opioid use disorder according to DSM-5 within the NMPOU group was diagnosed for 56.5% (n=13) and drug reports as well as hair samples revealed a clear dominance of opioid use compared to other substance classes (Table 1). One subject of the NMPOU group showed insufficient availability of hair samples to detect opioid metabolites; however, the urine toxicology was positive for opioids, therefore we decided to include the participant in the analyses. All other 22 individuals with NMPOU showed considerable opioid concentrations in hair samples, whereas hair concentrations of any psychotropic drugs in the control group were below established cut-off values (Cooper et al., 2012) except for one participant showing very low MDMA concentrations, which were considered as negligible. Twelve urine samples within the NMPOU group were tested positive for opioids. However, we decided to include them in the analysis and to consider potential acute effects in separate analyses.

4.4.2 Physiological stress response

Heart rate variability

T-tests revealed no significant group differences for RMSSD and log-transformed HF-HRV in both conditions (Table 2) even after controlling for BMI (p's>.05). However, paired-samples t-tests for controls revealed a significant increase of RMSSD from the inclusion to the exclusion condition (t(28)=-2.42, p>.05), which was also seen at a trend level in the NMPOU group (t(22)=-1.99, p=.056). Additional correlation analyses in the NMPOU group revealed strong negative correlation between opioid craving and HRV variables (p<.01, Table S1). Because of previously reported associations between craving and HRV in alcohol dependent patients (Ingjaldsson, Thayer, & Laberg, 2003; Quintana, Guastella, McGregor, Hickie, & Kemp, 2013), we divided the NMPOU group by a median split of the craving NRS ratings into "no/low craver" (LowC, n=15) and "medium/high craver" (HiC, n=8). Demographic characteristics and drug use are shown in Table S2. Based on the results of Ingjaldsson et al. (2003) and Quintana et al. (2013), we assumed that higher opioid craving was accompanied with lower HRV. Therefore, statistical analyses for HRV were carried out one-tailed. ANOVAs revealed statistical significance for RMSSD inclusion (F(2,49)=3.89, p<.05), HF-HRV inclusion (F(2,49)=3.89, p<.05), as well as for RMSSD exclusion (F(2,49)=5.33, p<.01), and HF-HRV exclusion (F(2,49)=7.34, p<.01) conditions with significant pair-wise comparisons specifically for HiC showing

the lowest vmHRV, whereas controls and LowC group showed no differences in vmHRV (Figure 1). Paired-samples t-tests revealed significant differences in RMSSD between both conditions only for the controls and for the LowC group (t(14)=-2.01, $p<.05$) but not for HiC (t(7)=-.43, $p=.341$).

Table 1. Demographic data and drug use (means and standard deviations)

	controls (n=29)	**NMPOU** (n=23)	**Value**	***df***	***p***
Female/male	10/19	6/17	χ^2= 0.42	1	0.515
Age	26.55 (8.1)	27.96 (10.3)	t = -0.55	50	0.582
Body mass index (BMI)	22.45 (2.6)	23.68 (3.6)	t = -1.42	50	0.162
Years of education	11.48 (1.5)	11.17 (2.0)	t = 0.62	39.20	0.542
Verbal IQ	105.24 (11.3)	106.39 (11.2)	t = -0.37	50	0.717
Employment (y/n)	28/1	19/4	χ^2= 2.87	1	0.090
BDI sum score	3.00 (3.3)	9.39 (7.7)	t = -3.71	28.35	**<0.001**
Cortisol hair concentration pg/mg	11.05 (12.5)	17.50 (20.6)	t = -1.82	50	0.075
Smoker/non-smoker	18/11	17/6	χ^2= 0.82	1	0.366
Cigerettes per week[a]	45.42 (35.7)	81.76 (57.9)	t = -2.20	26.35	**0.035**
Fagerström test (FTND)[a]	1.28 (1.6)	2.59 (2.3)	t = -1.92	28.06	0.065
Alcohol gram/week	69.65 (65.5)	51.28 (62.2)	t = 1.03	50	0.309
Opiates					
Times per week	-	3.88 (3.0)			
ME mg/week	-	543.35 (964.9)			
Years of use[b]	-	2.88 (0.5 - 28.0)			
Craving (NRS)	-	3.35 (2.7)			
Opioid withdrawal (OOWS)	-	0.25 (0.9)			
Positive urine tests (y/n)	0/29	12/11			
ME hair concentration pg/mg	1.32 (6.9)	4 318.2 (6 790.9)			
Cannabis					
Grams per week	0.07 (0.3)	0.36 (0.6)	U = 191.0		**0.023**
Years of use	3.73 (4.2)	4.78 (4.8)	U = 300.5		0.540
Positive urine tests (y/n)	0/29	5/18			
Amphetamine					
Lifetime gram[b]	0.00 (0.0 - 1.3)	0.00 (0.0 - 5018.5)			
Positive urine tests (y/n)	0/29	0/23			
Hair concentration pg/mg	0.00 (0.00)	19.57 (83.6)	U = 304.5		0.120
MDMA					
Lifetime gram[b]	0.00 (0.0 - 0.6)	0.10 (0.0 - 260.7)			
Positive urine tests (y/n)	0/29	0/23			
Hair concentration pg/mg	13.62 (59.9)	265.65 (505.3)	U = 228.0		**0.004**
Cocaine					
Lifetime gram[b]	0.00 (0.0 - 1.8)	0.10 (0.0 - 298.4)			
Positive urine tests (y/n)	0/29	1/22			
Hair concentration pg/mg	1.55 (8.4)	292.17 (557.3)	U = 195.0		**<0.001**

Significant *p*-values ($p<.05$) are shown in bold. T-test and Chi^2 for frequency distribution two-tailed.
[a]Median (range) is reported
[b]Only smokers
BDI: Beck's Depression Inventory, FTND: Fagerström test of nicotine dependence, ME: morphine equivalent, NRS: numeric rating scale (1-10), OOWS: objective opioid withdrawal scale (0-12).

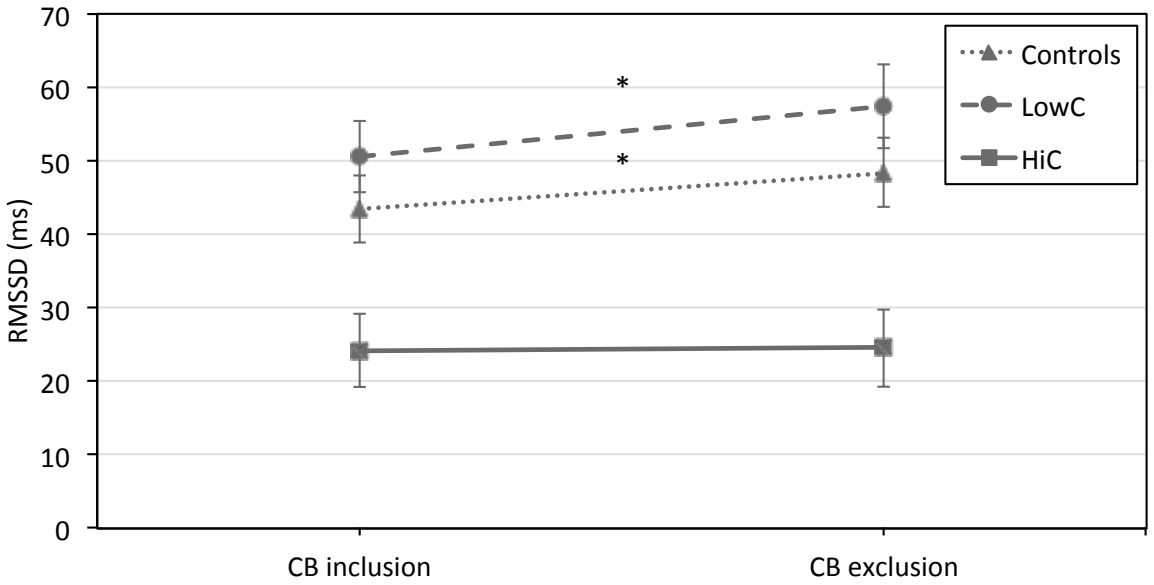

Figure 1. Average root mean square successive difference (RMSSD) in heart rate during inclusion and exclusion condition of the Cyberball task.
Paired-samples t-tests with $p<.05$*. Error bars reflected ±1 standard error.
CB: Cyberball, HiC: high craver, LowC: low craver.

Spontaneous fluctuation of the skin conductance

T-test analyses revealed no significant group differences in SF over the whole task and in the exclusion condition (Table 2). Additional ANOVAs with craving group as fixed factor and SF variables as dependent factors revealed again no group differences in SF of the SCL (Table S3) indicating similar response of the SNS over all groups.

Cortisol and ACTH

Repeated measure ANOVA for ACTH revealed no significant *group x time* interaction ($F(2.2,100.9)=1.78$, $p=.171$) and no *group* effect ($F(1,47)=1.47$, $p=.232$). Similar results were found for cortisol (*group x time* interaction: $F(1.9,92.9)=1.92$, $p=.154$); *group* effect: $F(1,48)=1.04$, $p=.313$). However, only the NMPOU group showed an increase of ACTH and cortisol after social exclusion (Figure 2), which was supported by significant group differences in ACTH and cortisol peaks corrected for baseline (T1) about 30 minutes after the Cyberball (Figure 2) and by a trend for the AUC_i cortisol ($t(48)=-1.73$, $p=.089$). Similar results were found for craving groups showing medium effects sizes for the AUC_i between controls and craving groups but not between LC and HC (Table S3).

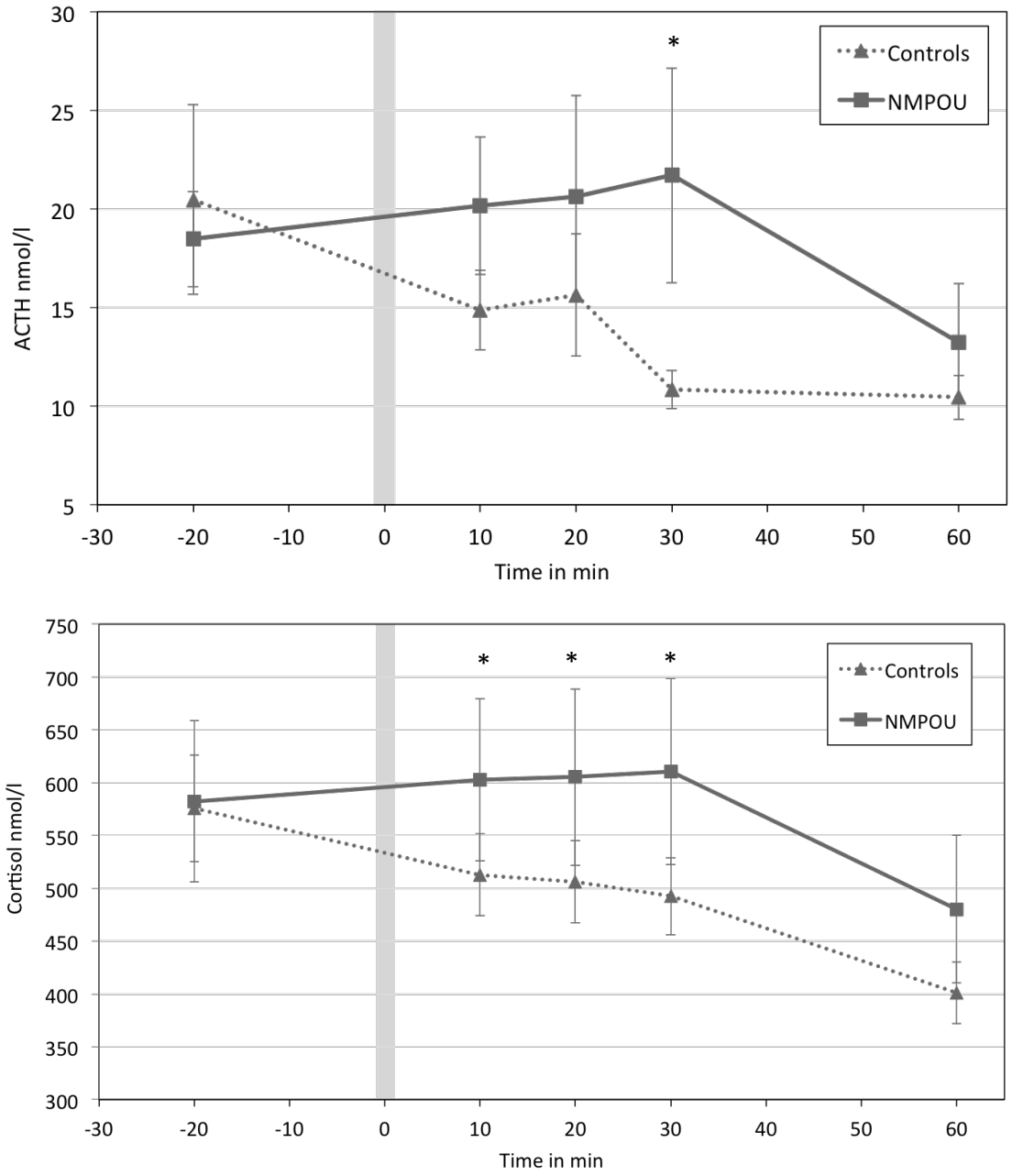

Figure 2. Mean ACTH (above) and cortisol (below) plasma concentrations before and after the Cyberball task Error bars reflected ±1 standard error. Shaded area highlights the duration of the Cyberball task. ANCOVAs for plasma samples corrected for baseline (t1) are indicated by $p<.05$*.

4.4.3 Behavioural assessments

PANAS and Cyberball questionnaire

Group differences were found for PA prior to the Cyberball (Table 2) indicating less positive mood in the opioid users. However, comparisons of PA and NA before and after the Cyberball task revealed decreased positive and increased negative state only for the controls (PA: $t(28)=2.227$, $p<.05$; NA: $Z=-2.088$, $p<.05$) but not for the NMPOU group (PA: $t(22)=.478$, $p=.637$; NA: Z=- -.317, $p=.751$). No group difference was found in the estimation of received balls during the game and in feelings of being excluded or included (p's>.05). These results did not change even after correction for age and

sex. HC revealed stronger feelings of exclusion compared to LC and controls. However, mood changes were only found in controls, whereas LC reported a slight decrease of NA after the Cyberball game (Table S3).

Social network size

The independent t-test revealed a significant smaller social network size (t(50)=2.39, p<.05) for individuals with NMPOU compared to controls (Table 2). The group effect remained significant even after correction for sex, age, and employment status (F(1,47)=6.22, p<.05) as unemployment might have an impact on social network size (Kroll et al., 2018). The ANOVA for craving groups revealed significance with a clear linear trend (p<.01), significant Sidak post hoc tests for controls and HiC (p<.05), and strong effects sizes (Table S3) suggesting a smaller social network specifically in HiC.

Table 2. Independent t-tests of dependent variables (means and standard deviations)

			Controls (n= 29)	NMPOU (n= 23)	t	*df*	*p*	Cohen's *d*
Physiological responses to social rejection								
vmHRV								
	RMSSD inclusion	*	43.36 (24.5)	41.34 (21.3)	0.31	50	0.756	0.09
	RMSSD exclusion	*	48.42 (25.2)	45.98 (25.4)	0.35	50	0.731	0.10
	log HF-HRV inclusion		6.34 (1.3)	6.26 (1.3)	0.23	50	0.820	0.06
	log HF-HRV exclusion		6.59 (1.1)	6.30 (1.3)	0.88	50	0.385	0.25
SF in SCL								
	SCL (µS)		3.02 (1.9)	2.91 (1.5)	0.23	50	0.818	0.07
	SCL exclusion (µS)		2.98 (1.8)	2.88 (1.5)	0.20	50	0.842	0.06
	DCM		0.12 (0.1)	0.12 (0.1)	0.01	50	0.995	0.00
	DCM exclusion		0.07 (0.1)	0.06 (0.1)	0.55	50	0.587	0.15
Behavioural assessments								
PANAS								
	PA pre CB	*	3.05 (0.6)	2.58 (0.5)	2.93	50	**0.005**	0.76
	PA post CB	*	2.74 (0.7)	2.56 (0.6)	1.05	50	0.298	0.29
	NA pre CB[a]	*	1.07 (0.1)	1.22 (0.3)	-1.82		0.068	0.60
	NA post CB[a]	*	1.19 (0.4)	1.25 (0.4)	-0.37		0.710	0.15
CB questionnaire								
	Feelings of exclusion		6.62 (1.5)	6.30 (2.2)	0.62	50	0.539	0.17
	Feelings of inclusion		3.45 (1.4)	2.83 (1.5)	1.56	50	0.126	0.43
	Estimated % balls received		16.28 (7.7)	16.65 (9.7)	-0.16	50	0.876	0.04
SNQ								
	Social network size total		21.31 (8.0)	15.83 (8.5)	2.39	50	**0.020**	0.64

Significant p-values (p<.05) are shown in bold.
Paired samples t-test (two-tailed) or Wilcoxon Signed-Rank test p<.05*
[a]Mann-Whitney U tests
CB: Cyberball, DCM: dynamic causal modelling, HF: high frequency, NA: negative affect, PA: positive affect, RMSSD: Root mean square of successive differences, SCL: skin conductance level, SF: spontaneous fluctuation, vmHRV: vagally mediated heart rate variability

4.4.4 Correlation analyses: morphine equivalents hair concentration and opioid use

Log-transformed ME hair concentration was not correlated with any stress response parameters of the ANS within the NMPOU group (*p*'s >.05). However, AUC_i ACTH was positively correlated with ME hair concentrations (r=.53, *p*<.01), which was also detected in AUC_i cortisol (r=.40, *p*<.05; Fig.3) even though with a smaller effect. Behaviouralπ data revealed no correlation with ME hair concentration. Duration of opioid consumption and positive opioid urine toxicology were not associated with social stress parameters (*p*'s >.05).

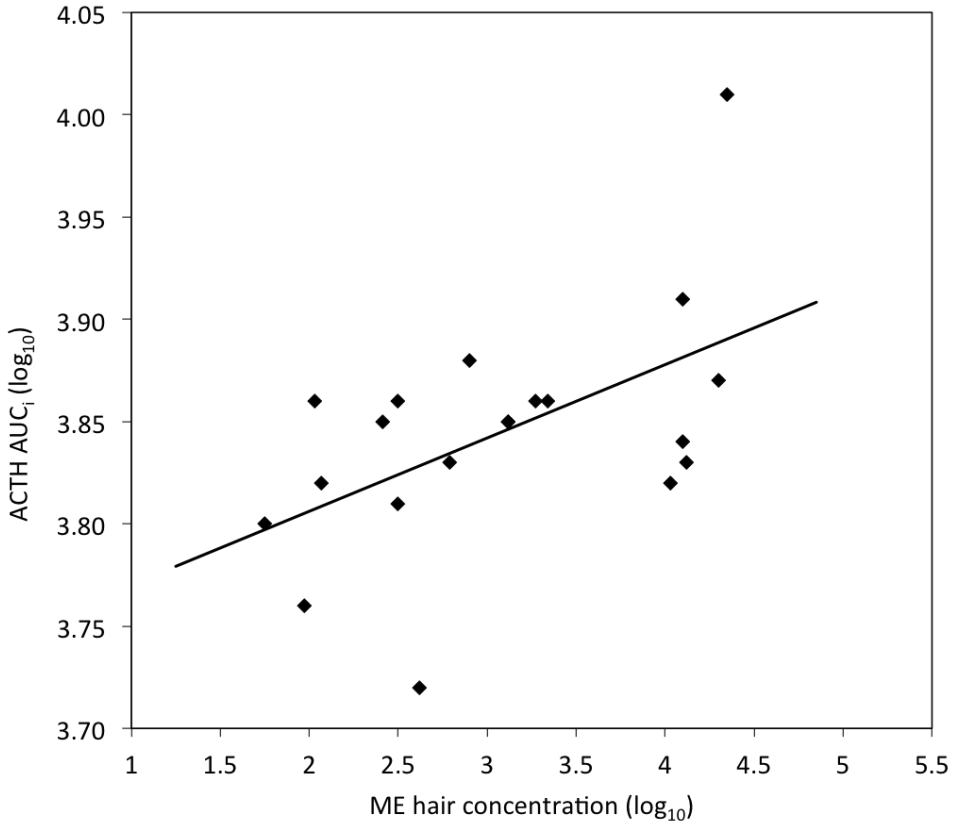

Figure 3. Scatterplot of the area under the curve to increase (AUC_i) for ACTH and cumulated morphine equivalents (ME) of opioid hair concentration. Pearson correlation analysis showed significant dose-dependent effects of opioids on ACTH AUC_i with *p*<.01.

4.5 Discussion

The aim of our study was to investigate potential effects of chronic NMPOU on behavioural and physiological stress responses to social rejection. We found that specifically high opioid craver (HiC) showed difficulties in emotion regulation during social rejection, whereas controls and low craver (LowC) revealed a better adaptation to psychosocial stress and emotion regulation indexed by vmHRV. Furthermore, no group differences were seen for SF of the SCL indicating preserved sympathetic activity in individuals with NMPOU. Interestingly, we found a pronounced increase of ACTH and cortisol for the NMPOU group compared to controls after the Cyberball suggesting a hyperreactivity of the HPA axis to social rejection, which was related to the opioid concentration in hair reflecting a dose-dependent effect. Furthermore, behavioural data indicated that individuals with NMPOU were aware of being excluded but less subjectively affected by social rejection than controls. Finally, NMPOU individuals revealed a smaller social network.

The main finding of our study was that individuals with NMPOU showed a dysregulated stress response to social exclusion specifically in the PNS and HPA axis. We found difficulties in emotion regulation during social exclusion for HiC indexed by lower and unchanged vmHRV during the Cyberball task. In contrast, LowC and controls showed an increased vmHRV during social exclusion suggesting adequate adaptation to social rejection. Our results are consistent with the findings by Ingjaldsson et al. (2003) reporting lower HRV for high craving alcoholics compared to controls. Low HRV has been associated with several psychopathological disorders such as depression, anxiety disorder, posttraumatic stress disorder, and alcohol dependence (Beauchaine & Thayer, 2015; Quintana et al., 2013). Furthermore, high vmHRV is associated with a better and more successfully emotion regulation of negative affect, whereas lower resting HRV was shown in individuals with difficulties in emotion regulation (Williams, Cash, Rankin, Bernardi, Koenig, & Thayer, 2015). Based on the Neurovisceral Integration Model (NIM) by Thayer and Lane (2009), the heart is under tonic inhibitory control of the PNS over the sympathetic nervous system (SNS) as indexed by vmHRV. Neuro-structural correlates of this process are represented by the inhibitory control of subcortical structures, such as the amygdala, via the PFC and its interconnection with the ACC and Insula (Thayer et al., 2012). These brain areas are also associated with emotion processes and social pain as well as with high opioid receptor density (Eisenberger, 2012; Nummenmaa & Tuominen, 2017). Furthermore, alterations of structural and functional connectivity of the amygdala, ACC, insula, and PFC were found in opioid dependent patients (Upadhyay et al., 2010; Younger et al., 2011). Consistent with these findings, the present results support the NIM and indicate that chronic NMPOU might lead to difficulties in emotion regulation and adaption to social rejection specifically modulated by craving. This is also consistent with a recent study reporting attenuated HRV response

during negative emotion regulation in chronic pain patients misusing prescription opioids (Garland et al., 2017). Participants were required to reappraise images with negative content as well as to savour and subjectively experience images with positive content. Interestingly, chronic pain patients misusing opioids showed no change in HRV from baseline to the reinterpretation of negative emotions, whereas non-misuser revealed an increase of HRV (Garland et al., 2017). Furthermore, prescription opioid misuser showed significantly higher craving scores than non-misuser and morphine equivalent daily opioid dose (MEDD) was not correlated with HRV, which is in line with the results of our study. In contrast to our findings regarding the PNS, results of the SCL indicated similar sympathetic responses between individuals with NMPOU and controls during social exclusion. This is coherent with a recent study investigating skin conductance response (SCR) to emotion-eliciting videos in opioid-substituted patients compared to controls reporting no group effects for the SCR (Biernacki, Terrett, McLennan, Labuschagne, Morton, & Rendell, 2018). Taken together, the present results of alterations in the PNS indexed by the vmHRV and no changes in the SNS indexed by the SCL in opioid users indicated that chronic opioid use might lead to a dysbalance within the ANS.

Analogously, chronic opioid use is commonly associated with dysfunction of the HPA axis reflected by suppressed glucocorticoid levels after opioid administration in animals and humans as well as by elevated cortisol levels during opioid withdrawal symptoms in heroin users (Kreek, Levran, Reed, Schlussman, Zhou, & Butelman, 2012; Vuong, Van Uum, O'Dell, Lutfy, & Friedman, 2010; Walter, Gerber, Kuhl, Schmid, Joechle, Lanz et al., 2013). Although our findings of equal ACTH and cortisol baseline levels between groups conclude that strong withdrawal effects as a potential confounding factor can be excluded in our study, we could not replicate the findings by Bershad et al. (2015) reporting attenuated cortisol response to psychosocial stress after administration of buprenorphine. In contrast, our results indicated an increased activity of the HPA axis only for the NMPOU group after the Cyberball task, whereas controls revealed no stress response to social exclusion. However, Bershad et al. (2015) investigated acute effects of a partial MOR agonist, whereas our study examined chronic effects of primarily full MOR agonists. Furthermore, social stress was induced by using the TSST, which might activate different biological stress response processes compared to psychosocial stress induced by social exclusion as conducted in our study. This is supported by the assumption that cortisol release is rather caused by stressors activating the need for power (i.e., mobilisation of stored energy) than the need for affiliation (Weik, Ruhweza, & Deinzer, 2017). Accordingly, some previous studies using the Cyberball paradigm in healthy participants were not able to find stress-induced cortisol increase after social exclusion (Bass et al., 2014; Jobst et al., 2015; Zwolinski, 2012). Therefore, non-response of the HPA axis in the control group of our study might be explained by this theory. However, individuals with NMPOU showed an increase of ACTH and cortisol

assuming a hyperreactivity of the HPA stress axis to social exclusion in chronic opioid users, which was also dose-dependent. Therefore, our results supported the findings of an opioid-induced dysfunctional HPA axis, albeit in a different direction. Given that animal studies reported inhibited production of endorphins and down-regulation of MOR after chronic opioid administration (Sprouse-Blum, Smith, Sugai, & Parsa, 2010) and that endorphins were postulated to inhibit and counteract over-activation of the HPA axis in response to stress (Bali, Randhawa, & Jaggi, 2015) our findings might be explained by: 1.) Chronic opioid use leads to down-regulation of the endogenous opioid release resulting in hypersensibility to social stress, which might be also a result from dysfunctional emotion regulation, and therefore entails maintenance of opioid use and opioid relapse. 2.) A lower baseline endorphin level entailing hypersensibility to social stress is a predisposing factor in individuals, who are prone to use opioids to reduce social distress. This view is supported by the fact that anxiety disorders including social phobia are more frequent in individuals with NMPOU (Becker, Sullivan, Tetrault, Desai, & Fiellin, 2008). Further studies should address these different causality explanations.

Behavioural data of the Cyberball revealed negative changes in mood after social exclusion only for controls, which is an indicator for the task's validity and supports the theory that psychosocial stress induced by social exclusion might activate different mechanism of stress response than stress-induction by the TSST. Although individuals with NMPOU showed a hyperreactivity of the HPA axis to social exclusion, no changes in mood was found after the Cyberball task. Furthermore, we could not replicate the findings by Bershad et al. (2016) reporting reduced perception of social rejection after buprenorphine administration. In contrast, our results revealed no group differences in the estimation of received balls and feelings of exclusion suggesting that individuals with NMPOU were aware of being excluded but nevertheless less emotionally affected by social rejection. Interestingly, HiC revealed stronger feelings of being excluded compared to LowC and controls, which might be resulted from a more pronounced dysfunctional emotion regulation in the most severe cases of NMPOU. These contrary findings might be caused by the fact that Bershad et al. (2016) investigated acute opioid effects and used a partial MOR agonist, whereas participants of our NMPOU group were asked to abstain from opioids on the testing day and showed chronic NMPOU of primarily MOR agonists. However, some urine samples of the NMPOU group were tested positive for opioids. Additional statistical analyses revealed no associations and differences of behavioural and physiological stress responses between individuals with NMPOU showing positive and negative urine toxicology for opioids. Given that the mean abstinence time for individuals tested positive for opioids was 14.3 hours (range 1-27 hours) and that the Cyberball task started about 2.5 hours after

measurement onset for the NMPOU group, positive urine toxicology might not represents acute effects in our NMPOU sample.

Assessments of real-life social functioning by the SNQ revealed a smaller social network size in individuals with NMPOU. Therefore, our findings support the BOTSA suggesting that exogenous opioids might replace the need for social contacts and decrease affiliative behaviour resulting in a smaller social network size and in being less emotionally affected by social rejection. Furthermore, the study by Johnson and Dunbar (2016) indicated an association between the endogenous μ-opioid system and social network sizes. However, a smaller network size was also reported in cocaine and polysubstance users (Kroll, Wunderli, Vonmoos, Hulka, Preller, Bosch et al., 2018c; Preller et al., 2014), which raise the question, if this phenomenon is caused by a predisposing factor, which might facilitate substance use, or whether chronic opioid use leads to a saturation of the MOR and therefore decreased affiliative behaviour resulting in less social contacts. However, several studies in animals but also in humans reported alterations of the endogenous opioid system after chronic cocaine administration indicated by an increase of MOR (Kreek et al., 2012). Therefore, the results of a smaller network size in cocaine but also in opioid users might supports the assumption that the endogenous opioid system plays a crucial role in affiliative behaviour and subsequently provide evidence for the BOTSA in humans. Longitudinal studies should clarify this question.

The study has some limitations: First, the sample size of the NMPOU group was small because of our strict exclusion criteria and limited prevalence of NMPOU in Switzerland. However, as a positive feature, our NMPOU group consists of relatively pure opioid users, objectively confirmed by urine and hair analyses. Furthermore, Cohen's *d* was calculated to address this limitation. Second, due to our study design, we were not able to compare behavioural, SCR, and neuroendocrine responses between inclusion and exclusion condition of the Cyberball. Therefore, we cannot unequivocally ensure that our results were distinct stress-related responses to social exclusion. However, affect changes after the Cyberball in the control group indicate that the task was valid. Third, cross-sectional designs do not allow interpretations regarding causality. Although our sample revealed relatively pure opioid use and less confounding factors such as pain disorders or psychiatric axis-I disorders, future longitudinal studies might elucidate the relationship between chronic prescription opioid use and response to social rejection more clearly.

To the best of our knowledge, this is the first study investigating effects of chronic opioid use on behavioural and physiological stress response to a psychosocial stressor such as social rejection. With respect to the opioid crisis in the U.S. and increased NMPOU also in Europe, it is important to

understand the underlying mechanism as well as the long-term sequelae of chronic opioid use. Our findings indicate that chronic NMPOU is not only accompanied with an abnormal functioning of the HPA axis but also with a dysregulation of the PNS. Individuals with high opioid craving revealed pronounced difficulties in emotion regulation and adaptation during social rejection indexed by vmHRV. Given that vmHRV is not only a downstream measurement but that HRV itself can affect emotion regulation and its brain networks, present findings might contribute to implement new treatments of opioid dependence such as HRV biofeedback to restore ANS balance (Mather & Thayer, 2018). This is supported by previous results of efficient HRV biofeedback in the treatment of heroin use with depressive symptoms and also of drug craving (Eddie, Kim, Lehrer, Deneke, & Bates, 2014; Lin, Ko, Fan, & Yen, 2016). Although subjective self-ratings in the NMPOU group implied that the perception of social exclusion was preserved, chronic opioid users were less subjectively affected by social rejection and reported a smaller social network than controls, which is consistent with the BOTSA and previous animal studies (Machin & Dunbar, 2011). Given that functional social support was found to prevent drug relapse and that inadequate stress response and craving are crucial risk factors of drug relapse, our results provide important implications for future interventions of opioid dependence targeting these deficits.

4.6 Supplementary material

4.6.1 Methods

Method S1 Procedure

Each test session started at 11:00 am for individuals with NMPOU and 11:30 am for the controls at the Psychiatric Hospital of the University of Zurich. Due to the circadian rhythm of the HPA axis, the Cyberball paradigm started around 1:30 pm for all participants as the afternoon was suggested as favourable for studying provoked stress response of neuroendocrine hormones (King & Liberzon, 2009). Participants were asked to have a balanced breakfast before arrival and to abstain from food and cigarettes during the measurements. Furthermore, participants were only allowed to drink water during the whole neuroendocrine assessments.

4.6.2 Results

Table S1 Correlation analyses between craving and HRV parameters

	Craving
RMSSD inclusion	**-0.49**
log HF-HRV inclusion	**-0.53**
RMSSD exclusion	**-0.48**
log HF-HRV exclusion	**-0.53**

Correlations coefficients with *p*-values $p<.01$ are shown in bold.

Table S2 Demographic data and drug use of the craving groups (means and standard deviations)

	Controls (n=29)	**LowC** (n=15)	**HiC** (n=8)	**Value**	***df***	***p***
Female/male	10/19	4/11	2/6	$\chi^2 = 0.43$	2	0.806
Age	26.55 (8.1)	26.20 (8.4)	31.25 (13.1)	F = 0.97	2,49	0.386
Body mass index (BMI)	22.45 (2.6)	23.67 (3.4)	23.70 (4.3)	F = 0.99	2,49	0.379
Years of education	11.48 (1.5)	11.53 (2.2)	10.50 (1.6)	F = 1.15	2,49	0.326
Verbal IQ	105.24 (11.3)	107.00 (12.1)	105.25 (10.0)	F = 0.13	2,49	0.881
Employment	28/1	13/2	6/2	$\chi^2 = 3.69$	2	0.158
BDI sum score	3.00 (3.3)	10.20 (8.6)	7.88 (5.8)	F = 8.52	2,49	**0.001**
ADHD-SR	8.97 (9.5)	16.53 (7.7)	12.88 (5.2)	F = 3.98	2,49	**0.025**
Physical pain (NRS)	1.31 (0.7)	1.60 (1.0)	2.75 (2.5)	F = 1.61[a]	2,14.23	0.234
Cortisol hair concentration pg/mg	11.05 (12.5)	13.14 (8.7)	24.36 (33.0)	F = 0.72[a]	2,16.18	0.502
Smoker/non-smoker	18/11	12/3	5/3	$\chi^2 = 1.54$	2	0.462
Cigarettes per week[b]	45.42 (35.7)	61.88 (48.1)	129.50 (54.8)	F = 7.49	2,32	**0.002**
Fagerström test (FTND)[b]	1.28 (1.6)	1.75 (1.9)	4.60 (2.3)	F = 6.78	2,32	**0.004**
Alcohol gram/week	69.65 (65.5)	53.30 (53.0)	47.50 (80.7)	F = 0.54	2,49	0.587
Opiates						
Times per week	-	2.81 (2.8)	5.88 (2.1)	t = -2.68	21	**0.014**
ME mg/week	-	544.42 (1166.0)	541.33 (455.0)	t = 0.01	21	0.994
Years of use	-	4.42 (6.8)	4.46 (2.9)	t = -0.02	21	0.987
Craving (NRS)	-	1.67 (0.9)	6.50 (1.9)	t = -6.96	8.8	**<0.001**
Opioid withdrawal (OOWS)	-	0.11 (0.3)	0.50 (1.4)	t = -0.77	7.46	0.468
Positive urine tests (y/n)	0/29	7/8	5/3	$\chi^2 = 20.40$	2	**<0.001**
ME hair concentration pg/mg	1.32 (6.9)	4 305.83 (7 861.1)	5 368.82 (5 768.6)	t = -0.33	20	0.742
Cannabis						
Grams per week	0.07 (0.3)	0.44 (0.7)	0.20 (0.4)			
Years of use	3.73 (4.2)	5.07 (4.3)	4.25 (6.0)			
Positive urine tests (y/n)	0/29	3/12	2/6			
Amphetamine						
Lifetime gram[c]	0.00 (0.0 - 1.3)	0.00 (0.0 - 5018.5)	0.00 (0.0 - 5018.5)			
Positive urine tests (y/n)	0/29	0/15	0/8			
Hair concentration pg/mg	0.00 (0.00)	3.33 (12.9)	50.00 (141.4)			
MDMA						
Lifetime gram[c]	0.00 (0.0 - 0.6)	0.15 (0.0 - 65.2)	0.00 (0.0 - 17.3)			
Positive urine tests (y/n)	0/29	0/15	0/8			
Hair concentration pg/mg	13.62 (59.9)	230.00 (483.1)	332.50 (572.6)			
Cocaine						
Lifetime gram[c]	0.00 (0.0 - 1.8)	0.30 (0.0 - 280.7)	0.05 (0.0 - 298.4)			
Positive urine tests (y/n)	0/29	1/14	0/8			
Hair concentration pg/mg	1.55 (8.4)	383.33 (644.2)	121.29 (308.3)			

Significant *p*-values ($p<.05$) are shown in bold. T-test and Chi^2 for frequency distribution two-tailed.

[a]Welch's test

[b]Only smokers

[c]Median (range) is reported

ADHD: attention-deficit/hyperactivity disorder, BDI: Beck's Depression Inventory, FTND: Fagerström test of nicotine dependence, ME: morphine equivalent, NRS: numeric rating scale (1-10), OOWS: objective opioid withdrawal scale (0-12).

Table S3 ANOVAs of the craving groups (mean and standard deviation)

		Controls (n= 29)	LowC (n=15)	HiC (n=8)	*F*	*df, df*	*p*	*p linear contrast*	Cohen's *d* controls vs LowC	Cohen's *d* controls vs HiC	Cohen's *d* LowC vs HiC
Physiological responses to social rejection											
HRV											
	RMSSD-HRV inclusion	* 43.36 (24.5)	* 50.50 (18.9)	24.16 (13.9)	3.89	2,49	**0.027**	**0.032**	0.31	**0.84**	**1.15**
	RMSSD-HRV exclusion	* 48.42 (25.2)	* 57.42 (22.4)	24.53 (14.9)	5.33	2,49	**0.008**	**0.013**	0.36	**0.95**	**1.31**
	log HF-HRV inclusion	6.34 (1.3)	6.77 (1.1)	5.30 (1.0)	3.89	2,49	**0.027**	**0.036**	0.34	**0.82**	**1.15**
	log HF-HRV exclusion	6.59 (1.1)	6.91 (0.9)	5.15 (1.3)	7.34	2,49	**0.002**	**0.002**	0.26	**1.19**	**1.45**
SF in skin conductance											
	SCL (µS)	3.02 (1.9)	2.65 (1.3)	3.41 (1.8)	0.54	2,49	0.587	0.577	0.22	0.23	0.45
	SCL exclusion (µS)	2.98 (1.8)	2.65 (1.3)	3.33 (1.8)	0.44	2,49	0.649	0.611	0.20	0.21	0.40
	DCM	0.12 (0.1)	0.13 (0.2)	0.09 (0.1)	0.26	2,49	0.776	0.609	0.11	0.21	0.32
	DCM exclusion	0.07 (0.1)	0.08 (0.1)	0.04 (0.0)	0.77	2,49	0.469	0.244	0.02	0.47	0.49
Plasma (nmol/l)											
	ACTH T1 (bsl)	20.48 (25.5)	21.92 (11.6)	11.56 (5.7)	0.65	2,49	0.528	0.311	0.07	0.44	0.51
	ACTH T2	14.88 (10.7)	22.88 (17.3)	14.68 (12.1)	1.89	2,49	0.163	0.972	0.60	0.01	0.62
	ACTH T3	15.63 (16.4)	23.93 (27.2)	14.00 (12.5)	0.98	2,49	0.383	0.845	0.42	0.08	0.51
	ACTH T4	10.82 (5.1)	24.60 (28.6)	15.90 (15.7)	3.18	2,49	0.051	0.476	0.79	0.29	0.50
	ACTH T5	10.45 (5.9)	14.70 (16.5)	10.30 (4.8)	0.92	2,49	0.408	0.973	0.43	0.01	0.44
	ACTH AUC_i	-547.52 (1595.0)	-9.76 (1220.2)	167.55 (545.7)	1.13	2,49	0.331	0.232	0.38	0.51	0.13
	Cortisol T1 (bsl)	575.74 (272.1)	617.36 (383.4)	512.23 (286.1)	0.27	2,49	0.762	0.627	0.14	0.21	0.35
	Cortisol T2	512.86 (210.1)	635.32 (334.0)	536.61 (402.5)	0.92	2,49	0.406	0.841	0.44	0.09	0.35
	Cortisol T3	506.22 (209.2)	633.54 (371.0)	548.43 (432.5)	0.87	2,49	0.426	0.737	0.43	0.14	0.29
	Cortisol T4	492.36 (196.0)	641.21 (360.9)	548.21 (502.7)	1.15	2,49	0.326	0.663	0.49	0.18	0.31
	Cortisol T5	401.09 (158.5)	496.63 (325.7)	447.21 (328.3)	0.75	2,49	0.476	0.652	0.40	0.19	0.21
	Cortisol AUC_i	-7136.18 (11276.2)	-733.3 (13779.0)	733.58 (15019.6)	1.87	2,49	0.165	0.143	0.50	0.62	0.12
Behavioral assessments											
PANAS											
	PA pre CB	* 3.05 (0.6)	2.55 (0.6)	2.63 (0.2)	4.25	2,49	**0.020**	0.074	**0.80**	**0.69**	0.12
	PA post CB	* 2.74 (0.7)	2.55 (0.7)	2.58 (0.5)	0.55	2,49	0.582	0.514	0.31	0.26	0.04
	NA pre CB[a]	* 1.07 (0.1)	* 1.17 (0.2)	1.33 (0.5)	3.40	2	0.183		0.38	**1.00**	**0.62**
	NA post CB[a]	* 1.19 (0.4)	* 1.10 (0.1)	1.53 (0.6)	4.62	2	0.099		0.23	**0.86**	**1.09**
CB questionnaire											
	Feelings of exclusion	6.62 (1.5)	5.67 (2.3)	7.50 (1.5)	3.07	2,49	0.055	0.214	**0.52**	0.48	**1.01**
	Feelings of inclusion	3.45 (1.4)	3.07 (1.6)	2.38 (1.3)	1.83	2,49	0.171	0.066	0.26	**0.74**	0.48
	Estimated % balls received	16.28 (7.7)	16.37 (9.6)	17.19 (10.5)	0.04	2,49	0.965	0.794	0.01	0.11	0.10
SNQ											
	Social network size total	21.31 (8.0)	18.07 (9.3)	11.63 (4.8)°	4.69	2,49	**0.014**	**0.004**	0.38	**1.13**	**0.75**

Significant *p*-values ($p<.05$) and medium to strong effect sizes are shown in bold.
[a]Kruskal Wallis test
Sidak post hoc test vs controls ($p<.05$)°
Paired samples t-test (two-tailed) or Wilcoxon Signed-Rank test $p<.05$*
ACTH: adrenocorticotropic hormone, bsl: baseline, CB: Cyberball, DCM: dynamic causal modelling, HF: high frequency, NA: negative affect, PA: positive affect, RMSSD: root mean square of successive differences, SCL: skin conductance level, SF: spontaneous fluctuation, vmHRV: vagally mediated heart rate variability